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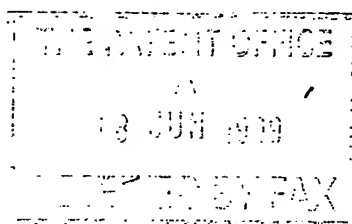
A. Brewer.

Dated

9 August 2000

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Patents Form 1/77

**The
Patent
Office**18JUN99 E455619-1 D02973
P01/7700 0.00 - 9914187.1**Request for grant of a patent****The Patent Office**
Cardiff Road
Newport
Gwent NP9 1RH

1	Your reference	MRH/P15770	18 JUN 1999
2	Patent application number	9914187.1	
3	Full name, address and postcode of the applicant	ML Laboratories Plc 17 Hanover Square LONDON W1R 9AJ 7436005001	
	Patents ADP number		
	State of incorporation	UK	
4	Title of the invention		
5	Name of agent	Harrison Goddard Foote	
	Address for service	Belmont House 20 Wood Lane Headingley Leeds LS6 2AE 14571001	
	Patents ADP number		
6	Priority applications	Country	Priority App No
			Date of Filing

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7	Parent application (eg Divisional)	Earlier Application No	Date of Filing
8	Statement of Inventorship Needed?		
9	Number of sheets for any of the following (not counting copies of same document)		
	Continuation sheets of this form		
	Description	5	
	Claims	-	
	Abstract	-	
	Drawings	1	
10	Number of other documents attached		
	Priority documents		
	Translations of priority documents		
	P7/77		
	P9/77		
	P10/77		
	Other documents		
11	I/We request the grant of a patent on the basis of this application.		
	Signature	<u>Harrison Goddard Foote</u>	Date 17 Jun 1999
12	Name and daytime telephone number of person to contact in the United Kingdom	Mr Michael R Harrison +44 113 2258350	

BIOLOGICALLY ACTIVE MATERIALS

Field of Invention

5 This invention relates to biologically active materials and, in particular, to materials which comprise a biodegradable polymer linked to a biologically active agent. The invention is concerned with materials known as polymer-drug conjugates which typically contain a therapeutic agent for instance, a bioactive cytotoxic drug, linked to a polymer back-bone. The linkage between the polymer and the drug is typically
10 by covalent bonding. However, the invention is applicable to other polymer conjugates including those where the biologically active agent is an imaging agent, such as tyrosinamide, a diagnostic agent, or a targeting agent such as biotin.

Reference will be made hereinbelow to polymer-drug conjugates in which the drugs
15 are anticancer agents. However, the present invention has application in connection with other drugs and/or bioactive agents.

Background of the Invention

20 In designing a polymer-drug conjugate, the aim is to deliver a drug effectively to a therapeutic site such as a tumour. It is known, for instance, that polymer-drugs given intravenously can accumulate selectively in solid tumour tissue by the EPR effect.

The most commonly used anticancer agents are low molecular weight compounds
25 which readily gain access to cells by rapid passage across the cell membrane. After intravenous (IV) administration, a large percentage of the injected dose leaves the circulation within a few minutes, resulting in a ubiquitous body distribution of drug and little selective concentration in tumour tissue. By creating a macromolecular polymer-anticancer drug conjugate, there is provided an opportunity to improve
30 tumour specific targeting, to minimise drug entry into sites of toxicity, to control precisely the rate of drug liberation at the target site (giving opportunities for long-

term controlled release) and to deliver the active principal intracellularly, thereby providing a means to overcome p-glycoprotein related multidrug resistance.

5 Numerous polymers have been proposed for synthesis of polymer-drug conjugates including polyaminoacids, polysaccharides such as dextran, and synthetic polymers such as N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer. However, these polymers have limitations. For example, a dextran-doxorubicin conjugate has been tested clinically and been found to be much more toxic than the parent drug. Furthermore the HPMA copolymers which have been clinically tested have the
10 disadvantage of being non-biodegradable in the main chain.

WO-A-98/56424 discloses a polymer-drug conjugate in which the polymer is the polysaccharide dextrin. Such a polymer-drug conjugate may be prepared in various ways. One method involves succinoylating dextrin and reacting the succinoylated
15 dextrin with the drug or a reactive derivative thereof.

WO-A-98/56424 includes an example in which the extent of succinoylation of dextrin varies from 2.26 to 6.64 Mol%. In a further example the drug doxorubicin is conjugated to succinoylated dextrans in which the extent of succinoylation varies
20 from 0.5 to 14.9 Mol%.

WO-A-98/56424 also includes examples showing the rate of degradation of dextrin both in the absence and in the presence of appropriate enzymes and also in rat plasma.
25

For at least certain applications the rate of degradation of dextrin in a dextrin-drug conjugate is an important consideration. For instance, it may be desirable to have a relatively slow rate of degradation in some applications while in other applications a faster rate of degradation is either acceptable or indeed even preferred.
30

Statement of Invention

It has now been surprisingly discovered that the rate of dextrin degradation is highly dependent on the degree of dextrin backbone substitution. As a result, it is possible
5 to tailor the dextrin by appropriate substitution of its backbone in order to achieve a desired rate of degradation.

More particularly, it has been found that, in the case of substitution of the dextrin backbone by succinoylation, relatively rapid degradation takes place at a degree of
10 succinoylation of up to about 15%. By contrast a degree of succinoylation above 30% very markedly reduces the rate of degradation.

The present invention provides a dextrin-drug conjugate in which the degree of substitution of the dextrin chain is greater than 15%, more preferably greater than
15 20% and most preferably greater than 30%.

The drug of the dextrin-drug conjugate may be loaded on the polymer via a linking group, such as succinoyl, in which case it may be attached to some or all of the linking groups. Alternatively the drug may be directly loaded onto the dextrin
20 backbone in which case the drug itself acts as the substituting group. As a further possibility the drug may be loaded partly via a substituting group and partly directly onto the dextrin backbone.

Brief description of the drawing

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The accompanying drawing is a graphical and tabular illustration of the degradation of dextrans of different degree of modification by α -amylase.

Detailed description of the invention

5 *Example 1*

Dextrin (Mw 51,000 Da) was succinoylated using a modification of the method described by Bruneel *et al* (Polymer, 35 (12),(1994), 2656-2658). Doxorubicin was then conjugated directly via an amide bond, conjugated via an N-*cis*-aconityl spacer or conjugated via a glycy-N-*cis*-aconityl spacer.

10

Polymer degradation (unmodified dextrin, succinoylated dextrin (5, 15 mol %) and conjugate) was measured in the presence of amylase or lysosomal enzymes to monitor either changes in polymer molecular weight (GPC) or doxorubicin release (HLPC).

15

The dextrin-doxorubin conjugates had a doxorubicin loading of 6-12 wt% dependent on the reaction conditions used and the degree of succinoylation of the dextrin intermediate. Table 1 shows the characteristics of several batches of dextrin-succ-doxorubicin

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Table 1 Characteristics of batches of dextrin-succ-doxorubicin

Batch No	Dox	
	(wt%)	Free Dox (% total Dox)
1	11.7	0.8
2	11.9	2.0
3	8.7	1.2
4	8.4	0.1

25

30 After a 180 min incubation with amylase, unmodified dextrin is almost completely degraded to low molecular products, whilst the succinoylated dextrin (5 and 15 mol %) and dextrin-succ-doxorubicin show a biphasic pattern of degradation giving rise to fragments of Mw 4,000, 9,500 and 6,400 Da respectively. Unmodified dextrin had

a $t_{1/2}$ (time for mass to reach half of its original) of 20 min, succinoylated dextrin and dextrin-succ-doxorubicin a $t_{1/2}$ of approximately 15 min.

Example 2

5

In this example the degradation of dextrans of different degrees of modification was compared. The results are shown in the accompanying drawing. It will be seen that native dextrin is rapidly degraded as are also dextrin with 5% succinoylation (whether with or without 6% Dox) and dextrin with 15% succinoylation. However, 10 if dextrin is 34% succinoylated the degree of degradation is markedly less, there being zero% reduction of the peak mass of primary peak after 60 minutes and only 20% reduction after 180 minutes.

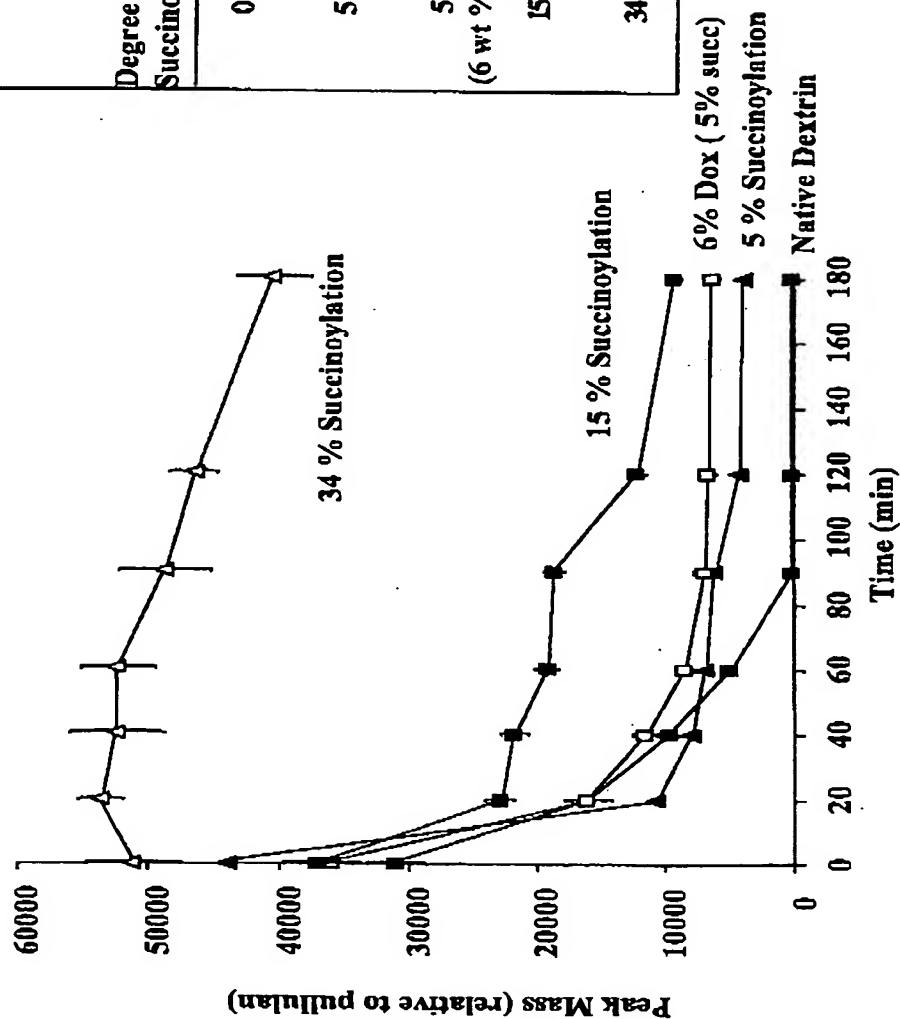
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Degradation of Dextrins of different degrees of modification by α -Amylase



$n=3 \pm S.D.$

Degree of Succinoylation	% Reduction of peak mass of primary peak	
	60 min	180 min
0	84	99
5	86	90
5 (6 wt % Dox)	77	92
15	49	75
34	0	20

- Dextrin degradation by amylase is dependent on the degree of backbone succinoylation. The dextrin-dox conjugate is rapidly degraded to oligomers

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